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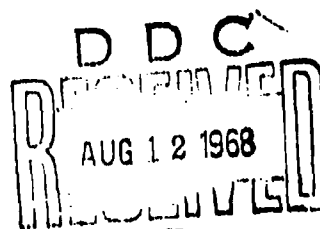
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The virulence and pathogenicity of cowpox virus.

by H. Moritsch.

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One hundred and sixty years ago, on May 14, 1796, Edward Jenner carried out that historically well-known attempt to use pustular material from the hand of a girl who had contracted genuine cowpox while milking to inoculate an 8-year-old boy in order to protect him against a subsequent infection with smallpox. Six weeks later the boy could no longer be infected with variola, offering the first proof of the protective effect of cowpox vaccination against later smallpox. The fact that a cowpox infection could protect a person against smallpox had been known to the rural population for some time and probably influenced Jenner's decision and action, as may be gathered from Kaiser's (1949) excellent presentation of the history and development of smallpox vaccination. These reports as well as the literature references listed in Eberbeck's (1939) Manual of Virus Diseases demonstrate further that the concept of "cowpox infection" was known in those days specifically as a separate symptomatology in cows, including its transmissibility to man.

This clear concept of "cowpox" later became confused in proportion to the efforts of various countries to immunize their population against smallpox. In keeping with Jenner's ideas, original plans included vaccination with genuine cowpox virus and the ultimate establishment of separate institutes for this purpose, which would maintain this vaccinal virus at constant virulence and would use it to produce a workable vaccine. The literature employed the term "cowpox virus" when referring to this vaccinal strain, until Downie (1939) called attention to the fundamental difference between genuine, original cowpox virus and the vaccinia virus utilized. This led to the justified question whether the vaccinia virus employed as immunogen was descended from genuine cowpox virus and had merely suffered changes during constant cultivation at the immunological institutes, or whether another strain of the pox virus group should be considered the parent of this vaccinia virus. However, since all attempts to confer the properties of vaccinia virus on genuine pox virus or alastrim strains or on another strain of the animal pox group have failed in various passage experiments, this question must remain unanswered for the time being. The problem of a possible "primordial strain" of the

entire pox virus group similarly rests only on assumptions. If the problem of origin of the individual pox group strains is set aside, and discussion is limited to factual demonstrations of differences between the various strains of this group, it must be conceded that there is no longer an identity between cowpox virus and vaccinia virus. For this reason the designation "cowpox virus" should be reserved for the pathogen of original cowpox, and the immunogenic strains should be known as "vaccinia virus." Demonstrated for the first time by Downie's studies, this discovery was supported and developed in subsequent years by variegated investigations, until it has become possible to fix the differential diagnosis of a cowpox virus infection without great difficulty in a suitable laboratory. These viral properties involve the behavior of the virus in the cell and in the tissue, considered morphologically characteristic of cowpox virus due to its unusual pathogenicity. Seroimmunological tests also permit clear differentiation of this virus. Since our own studies have uncovered new aspects of the problematic complex of virulence and pathogenicity of cowpox virus, it seems indicated to present a compilation of current intelligence.

For reasons of perspicuity, the present paper will first discuss generally valid properties of the virus, i.e., formation of inclusion bodies and the characteristic appearance of the local infection, followed by the antigenic structure of the virus in correlation with immunity and serological techniques, including known interference phenomena, and will close with the peculiar pathogenicity of the virus in man, cattle, rabbit, mouse and hen's egg. ( ) ←

#### Formation of inclusion bodies by cowpox virus.

When a tissue section infected with cowpox virus is stained with hematoxylin-eosin and observed under medium power, initially recognizable factors are a) the size and location of inclusion bodies and b) the hemorrhagic nature of the local infection. These two characteristics are particularly marked when a tissue section analogously infected with vaccinia virus is prepared for comparison. Vaccinia virus also tends to form inclusion bodies in the cytoplasm of a proliferated cell with ballooning, vacuolated or reticulated degeneration, with the difference that inclusion bodies are not always demonstrable under the light microscope and invariably have a minute size, so that the nucleus of the affected cell usually is still visible next to the inclusion body. The exact structure of these vaccinia virus inclusion bodies are shown on electron micrographs by Gaylord and Melnick (1953). It is evident that these inclusion bodies consist of numerous individual particles seemingly held together by a matrix. Based on these studies, the authors differentiate between "immature" and "mature" virus particles. In contrast to these inclusion bodies due to vaccinia infection, now well-known even with respect to their fine structure, the inclusion bodies of cowpox virus infections are marked by their unusual size. They frequently occupy the entire volume of the displaced cytoplasm and it seems as if

the cell nucleus has been compressed into a crescent against the cell wall. This singular size is so conspicuous that Kaiser and Gherardini (1940), independently of Downie's studies, referred to this phenomenon in connection with a strain of the pox group and called attention to the dissimilarity in size when compared to vaccinia virus inclusion bodies. They noted granulation in these inclusions and assumed that Guarnieri bodies consist of viral colonies held together by a binding substance. The ability of cowpox virus to produce particularly large inclusion bodies seems to be subject to fluctuation, the cause of which might be found in the changing relationship between virus and cell. Berger (1956) believes that this property will be lost eventually when the strain is carried through rabbit corneal passages in the laboratory. He assumes that unusually large inclusion bodies may be expected only in those cells where central cellular necrosis proceeds with particular slowness, allowing sufficient time for outstanding development in size. This would be true most of all when the infectious material is transmitted directly from the cow to the test animal. The reaction is said to be quite protracted in the first test animal passage, while increasing adaptation to the new host not only shortens the incubation time, but also causes the inclusion bodies to become proportionally smaller. Our own experience shows, however, that a strain of cowpox virus continued in the laboratory had apparently lost this ability to form inclusion bodies after one human passage upon recultivation on the rabbit cornea and subsequent transfer to the chorioallantoic membrane (CAM). The large inclusion bodies reappeared, however, in their usual scope in the 4th CAM passage, suggesting the possibility of reversible processes (Doseh and Moritsch 1956).

The localization of inclusion bodies and the site of viral multiplication must be sought principally in the ectoderm, although the infection may start in the endoderm and mesoderm, so that one cannot speak of a strict tropism of this virus, but rather of a preference for one germinal layer -- the ectoderm -- so clearly demonstrated in the hen's egg and in other animal tests.

#### Local infection with cowpox virus.

The most conspicuous sign of a cowpox infection is the hemorrhagic nature of inflammatory infections in the tissue. This extraordinarily effective component leads to edema with hemal congestion and hemorrhage in the connective tissue. It is demonstrable in the organism in all infective tests, regardless of the test animal and the site of infection, and is described unanimously by all authors. It differs from hemorrhages associated with testicular and nerve virus, since it is found not only in rabbits but also in the egg (Herrlich and Mayr 1954).

This assertion is qualified, however, by observations made by van Tongeren, who succeeded in isolating so-called "cowpox virus mutants" from typically hemorrhagic cowpox strains. When the egg membrane is inoculated with an original strain of cowpox virus, incubation for 3 days

will produce many "red" pox and a few "white" pox. The isolation of this white mutant for purposes of pure culture succeeds after 5-8 egg membrane passages, resulting in a pure strain of cowpox which produces only such "white" pox on the egg membrane. This mutant of cowpox virus, obtained by selection, is still capable of producing local infection with large inclusion bodies on the egg membrane, but without hyperemia and hemorrhages; in monkeys, rabbits, guinea pigs and mice, on the other hand, local and generalized reactions to inoculation are very weak or entirely absent (van Tongeren 1952). Nevertheless, this variant must not be considered a new virus strain of the pox group, discovered in a mixed infection with cowpox virus during transmission to the egg membrane, but must be judged a genuine mutant of cowpox virus, since

1. it is always possible to isolate such mutants from pure cultures of cowpox strains,

2. the double-diffusion test developed by Gispén (1955) failed to show a difference in antigenic structure between original "red" and selected "white" strains of cowpox virus, and

3. inoculation of calves with "white" mutants confers immunity against genuine "red" cowpox (van Tongeren 1952).

#### Antigenic structure of cowpox virus and seroimmunological studies.

Aside from the characteristics of a local infection with cowpox virus, the latter may be differentiated positively from variola and vaccinia virus by exact seroimmunological tests. Initial studies were carried out by Downie (1938, 1939). In comparisons with vaccinia virus he found first that agglutination tests with elementary body suspensions and complement fixation reactions (based on methods employed by Craigie and Wishart in 1934 in connection with vaccinia virus) produced much higher serum titers with homologous virus than with heterologous virus. Following absorption of immune sera with homologous elementary bodies, he was unable to demonstrate antibodies in agglutination tests, in OFR and in neutralization tests either against the homologous or the heterologous strain. On the other hand, absorption with heterologous elementary bodies removed only antibodies directed against this (heterologous) virus strain from the immune serum, indicating that the latter could be induced to produce a specific antigen-antibody reaction with the original (homologous) virus strain, even though on a reduced level. Downie, however, stressed already in 1939 that a precise determination of pure "L" and "S" antigens would bring further information on the degree of consanguinity between cowpox and vaccinia virus. Additional studies by Downie and coworkers (1950) show that this method permits differentiation of vaccinia and variola virus from cowpox virus, but that the latter could no longer be differentiated from ectromelia virus. Similarly, the double-diffusion test developed by Gispén for the

differentiation of pox group viruses permits only distinction between variola-vaccinia virus on one side and cowpox (including the "white" mutant)-ectromelia virus on the other. Differentiation between cowpox and ectromelia virus is not possible with serological methods and must be made in animal tests.

This variable action of vaccinia and cowpox virus in serological reactions was confirmed or supplemented by personal neutralization tests. These differed from preceding studies to the extent that they represented the first instance in which a vaccinia virus strain (Vienna) adapted to the mouse brain and an original cowpox virus strain were titrated in cross-tests by intracerebral infection of mice weighing 8-10 g with rabbit immune sera. In these tests the intracerebral infection of the mouse proved more sensitive than intracutaneous application to the rabbit back, as already pointed out by Haagen (1934) in connection with his attempts to adapt vaccinia virus to the mouse brain. In this manner a more strongly neutralizing effect of cowpox immune serum was obtained, in comparison to vaccinia virus immune serum against the strain of vaccinia virus (Dosch and Moritsch 1956).

All these reports were concerned with comparisons of cowpox virus with other strains of the pox group in serological tests. An exact analysis of the antigenic structure of cowpox virus has been attempted heretofore only by Mayr, Herrlich and Mahnel (1955). They based their studies on the hypothesis that the total antigenic complex of cowpox virus contains different individual antigens:

1. V-antigens linked to the viral elementary unit,
2. a) S-antigens, b) hemagglutinins separable from the viral elementary unit, and
3. a host-specific normal component antigen (NK-antigen) which exists both in solid linkage with the virus and in separately demonstrable form.

Exact data on S-antigen exists today only with respect to vaccinia virus.

This S-antigen is always found at the site of viral propagation, although its role in viral synthesis is not clear. It is non-infectious and therefore cannot by itself immunize against the complete virus, although it leads to the formation of specific antibodies against S-antigen. This S-antigen may then be prepared in a specific manner and fixed in the presence of complement. The literature dealing with vaccinia virus makes a distinction between a thermolabile "L"-component and a thermostable "S"-component, both of which are in solution as "LS"-complex and are said to represent a portion of the surface of vaccinia virus.



In the course of their extensive work these authors found that purified, virus-specific S-antigen of cowpox virus was thermostable and that, contrary to preceding studies by other authors with vaccinia virus, no thermolabile L-component could be found either in vaccinia virus or in cowpox virus. They concluded that the so-called L-component is not a constituent of "LS"-complex (coined in connection with vaccinia virus), but represents a host-specific normal component always present in large amounts in unpurified S-antigen starting material.

Thus no principal difference in the structure and composition of the antigenic complex seems to exist between cowpox and vaccinia virus, permitting comparisons -- in this sense -- between the two strains. However, it must be stressed again that there are quantitative and, in part, qualitative dissimilarities. The extent to which this is expressed in the immunogenic property of a cowpox virus strain has not been examined sufficiently in comparative tests. Our own studies merely show that a rabbit infected with vaccinia virus may be reinfected corneally with cowpox virus, while a rabbit infected initially with cowpox virus is immune against a secondary infection with vaccinia virus (Dosch and Moritsch 1956). This indicates that infection with cowpox virus may confer a higher degree of immunity than infection with vaccinia virus.

#### Interference with cowpox virus.

Since the interferential phenomena connected with viral infections attract special attention, we shall refer to the only two phenomena known from cowpox infections. Dosch (1955) succeeded in establishing the phenomenon of a secondary auto-interference in virological and histological studies by infecting hen's eggs with an original strain of cowpox which was well adapted to the egg. He infected the CAM twice locally at intervals of 3 days and observed, both macroscopically and microscopically, a reciprocal action in the sense of developmental inhibition in the local infection. The inhibiting effect of the first infection on the second was clearly indicated, while a simultaneous effect in the opposite direction could not always be demonstrated. Moreover, the development of inclusion bodies in the tissue was nearly parallel to the macroscopic findings. Since these tests employed two infections with the same viral strain, the designation of the phenomenon as a secondary auto-interference seems justified.

Personal studies revealed further that the phenomenon of foreign interference with a neurotropic virus may be produced in cowpox infections of mice. The interference observed in this case was foreign, since the reciprocal influence of two different virus strains was involved. Histological examination of mouse organs for the first time revealed pathological changes which had not been seen heretofore upon separate infection with each of these two strains. These processes are being studied in tests now in progress.

## Pathogenicity of cowpox virus.

### 1. Human infections.

Local infection of man with cowpox virus leads to formation of vesicles and pustules on the skin in a fashion typical of poxvirus strains. This local infection may be differentiated macroscopically from vaccinia virus infections by its special hemorrhagic component. The epithelial cells of the skin reveal paranuclearly situated inclusion bodies. The virus may be recultured from the pustular contents in the customary manner.

Whether the assumption repeatedly cited in the literature, that cowpox virus produces a "milker's nodule" on human skin, is still valid today, must be confirmed positively by newer investigations. The last experimental studies and observations by Berger (1955, 1956) indicate that no virological or seroimmunological correlations exist between cowpox virus and milker's nodule. Personal studies (not published) based on histological examinations of local cutaneous infections have shown an etiological connection between milker's nodule and cowpox virus to be improbable, since the pathological reaction involved does not resemble a cowpox infection and no virus of the pox group has ever been isolated. This raises the question, whether the concept of "milker's nodule" should be generalized and whether this might possibly represent a genuine cowpox virus infection, which might lead to constant confusion, or whether an infection sui generis is involved (an unproved assumption), which leaves the concept of "milker's nodule" in a strictly separate category, certainly not synonymous with a cowpox virus infection. It no longer seems feasible to speak of "milker's nodule sensu strictiori" and to use the term milker's nodule (citing Kaiser 1952) for four etiological and clinically different, but similar, morbid processes. Rather, one should try to separate the concepts of "cowpox" and "milker's nodule" once and for all on the basis of their differing etiology, now that exact studies of cowpox virus have made it possible to differentiate the latter as a separate strain of animal pox.

Similarly, contagious pustular dermatitis of sheep, popularly known as ORF, as mentioned by Bieling (1954), a disease transmissible to cows and human beings, should be compared to milker's nodule and should be differentiated from a genuine cowpox virus infection.

As indicated by Jenner's studies, genuine cowpox infections of cattle and man have been known for some time from their clinical aspects. Detailed reports on human infections with isolation of the virus have come from England (Downie et al. 1938, 1951) and Holland (Verlinde 1951). These infections are found in agricultural workers who contract cowpox virus infections by contact, especially during milking. However, not every infection with a virus of the pox group transmitted from cow to man should be considered a cowpox virus infection, since a genuine "vaccinia inoculata" may be encountered occasionally, as shown by investigations

by Otte and Mochmann (1955). For this reason, the diagnosis of "cowpox virus infection" should not be geared solely to demonstration of the infective route cow-man, but ought to depend exclusively on a successful differentiation of the isolated pathogen.

A frequent complication observed in infants is the smear infection of the eye, leading to secondary vaccinia efflorescences, preferably on the rim of the eyelid. This complication has been described also in connection with cowpox infections (Cruickshank 1910, Mader 1919, Ffingst 1941). All of these cases involve infections with rather benign courses, similar to the usual complications with vaccinia virus. A single case deserves to be pointed out, since it involved a virulent laboratory infection (Doseh and Moritsch 1956).

This infection was contracted during manipulations of enriched virus, which squirted under pressure into the right eye of the patient who had been vaccinated last 20 years ago. Within 14 days, the entire conjunctiva bulbi developed an ulcerous process with chemosis and edema which ultimately led to reversible clouding of the cornea. In addition, there were secondary efflorescences on the rim of the eyelid and the nose, both internally and externally. The virus was recultured from the conjunctiva on the 12th and 14th day after infection, and typical inclusion bodies were demonstrated in the epithelial cells of the conjunctiva. Following a 30-day stay in the hospital, the inflammatory edema had subsided enough to permit the patient's discharge. Complete restitution, marred by weak, irregular astigmatism, set in after 10 months (Doseh and Moritsch 1956, Pillat).

Another singular case involves isolation of a cowpox strain from the pustular content of a child affected with so-called "eczema herpetiforme Kaposi" (Doseh and Moritsch 1956, Tappeiner 1954). This process could have been designated also as "eczema vaccinatum," but it seems proper, for didactic reasons, to use this term only for the complication developing in the wake of vaccination with vaccinia virus, and to employ the term coined by Kaposi (1893) for the broader clinical syndrome requiring identification of the pathogen by virological means.

A particularly severe and fatal complication of a local infection with cowpox virus was reported from Holland (Schreuder, van Rijssel and Verlinde 1950, 1951).

A 15-year-old girl who had never been vaccinated developed encephalomyelitis 14 days after a local infection of her hands due to milking of infected cows. Cowpox virus was isolated from the cow's udder and from the girl's hands, but not from her brain upon autopsy. It is noteworthy that histological examination of the brain revealed the same pathological changes as those found in so-called postvaccinal encephalitis of man.

This finding shows, therefore, that

1. the morbid symptoms of so-called human postvaccinal encephalitis may be provoked by infection with another virus of the pox group,
2. Jenner's old method, requiring vaccination with genuine cowpox virus, offers no guarantees against complications.

This case is of primary importance for all subsequent experimental research into the causes of so-called postvaccinal encephalitis, since it involves a human case and indicates that such tests are possible not only with vaccinia virus, which is not always readily demonstrable, but also with cowpox virus, which tends to form large inclusion bodies and is more readily adapted to different test animals (Moritsch 1956; van Tongeren 1952).

## 2. Infections of the mouse.

The mouse lends itself readily to the study of cowpox virus infections, because

1. it is possible to apply virus in different ways (i.e., i.v., i.p., s.c.),
2. the mouse is very sensitive and reacts differently to the various routes of infection,
3. demonstration of virus in the mouse is relatively easy both virologically and histologically,
4. even large-scale experiments are not too expensive.

The first infective tests date back to van Tongeren, who succeeded regularly in killing mice through i.c. and i.v. infection and in growing the original cowpox strain from the material (van Tongeren 1952).

It was shown later, in extensive tests of our own, that infection with our own strain of cowpox leads to death even after s.c. infection, in contrast to the vaccinia virus strain "Vienna," adapted to the mouse brain, which is 100% lethal only upon i.c. application to mice weighing 8-10 g.

The clinical course of a mouse infected i.c. with cowpox virus is peculiar and resembles that described by Rosenau and Andervont (1931) in connection with mice infected with vaccinia virus. It differs considerably from the appearance of an infection with a pronounced neurotropic virus strain. Paralysis is never seen, but attacks of cramps setting in shortly before death have been noted, accompanied by rhinitis and severe conjunctivitis. The mice huddle closely together,

their fur bristles and their pale tails have bamboo-like contractures. Mice infected with cowpox virus by different routes (i.p., s.c., i.v.) show a similar picture, with the important difference that cramps are never seen after i.c. infection. Cowpox virus shows maximal settlement in the brain 3 days after i.c. infection, while all other routes induce the highest viral content in the liver and spleen. Thus, in contrast to a neurotropic virus strain adapted to mice, it is impossible to show spontaneous viral multiplication in the brain after non-i.c. infection, nor do passages produce such adaptation of the virus to the mouse brain, as usually obtained from freshly isolated neurotropic strains. Perhaps the main reason is to be found in as yet unknown reactions taking place upon invasion of a cell by virus, at the site of virus propagation in the brain, which may be linked to the type of cell in which viral multiplication occurs. Thus, neurotropic viruses multiply in the ganglion cells of the CNS, leading to histological pictures of frank encephalitis with neuronophagia, perivascular cellular infiltrates, meningitis and others, while cowpox virus introduces intracerebrally attacks principally the epithelial cells of the plexus chorioideus and the ependyma of the ventricles. Such a cerebral section, when stained in the customary manner with hematoxylin-eosin, may look quite innocent, unless the plexus chorioideus is observed with Mann's stain, which visualizes inclusion bodies particularly well and the pathological changes are revealed in preparations stained by other agents. It is quite understandable that viral affection of these different brain cells may be triggered by different factors. Our own studies have shown that peripherally applied (not i.c.) infection of the mouse with cowpox virus cannot induce viral affection of brain cells either with the aid of traumatic irritants (i.c. injection of sterile solution) or by simultaneous application of different drugs (cortisone, trypan blue, cardiazol, theophylline). On the other hand, i.v. infection and daily treatment of the mouse with electric shock (1 min. 70 volts AC) produced viral multiplication in the brain, demonstrable both virologically and histologically (Moritsch, in print). It must be stressed, however, that inclusion bodies were found only in the purely mesenchymal part of the brain after these provocations, i.e., in the meninges and in the stroma of the plexus chorioideus, indicating a difference between the infective mechanism of a direct i.c. infection and one provoked by electric shock.

A peculiar and heretofore unmentioned phenomenon is the reciprocal interaction of a neurotropic virus strain (group of the Russian early summer encephalitis viruses) with our own cowpox virus after peripheral infection of the mouse. Since histological studies of mouse organs had failed to disclose lesions typical of these two strains, a foreign interference manifestation seemed to be involved, whose mechanism is as yet unknown, since serial studies are still in progress.

Histological examinations of mice infected variously with cowpox virus yielded inclusion bodies in different organs. These formations were found regularly at the site of infection. e.g., in the epithelial cells of the skin after intracutaneous infection, in the epithelial cells of the plexus and the ventricular endyma upon intracerebral application, in the alveolar epithelium of the lungs after i.n. infection, etc., but appeared also in various distant organs due to hematogenic dissemination, as in the suprarenals, testes and elsewhere. On the other hand, the liver reveals characteristic necrosis which may attain the size of pinheads, particularly after i.v. infection. It is noteworthy, moreover, that no inflammatory cellular defensive reactions are seen and that the transition of necrosis to healthy liver parenchyma is formed only by numerous cells with inclusion bodies, cells that have not been destroyed as yet. Since the multiple necroses are always found in the mouse liver, even after variable infective techniques, they represent a valuable clue to morbid histological examination of mice in the differential diagnosis of cowpox virus infections.

### 3. Infections of the rabbit.

The use of rabbits as experimental animals for the demonstration of pox group viruses is based on long experience. Its special advantage arises primarily from the circumstance that these animals may be infected outaneously and that the local reaction on the skin permits a quantitative determination of viral content as well as neutralization tests. In addition, infection of the scarified cornea (Paul) and the testes (Othawara) is an excellent means for the isolation of a virus strain from questionable material.

Herrlich and Mayr (1954) have given very thorough details of the course of an intracutaneous infection with cowpox virus and the overall symptoms developing from it, including generalization. These authors initially found only very flat local infiltrations, which were accompanied after 48 hours by petechial hemorrhage. These extravasations increase and cover the whole infection during the next 48 hours. Now central fusion of tissue sets in, delimited proximally by an anemic zone of irritation and peripherally by a deep hemorrhagic region which gradually blends with the healthy tissue surrounding it. Severe edema is seen in the subcutis. This type of local hemorrhagic inflammation is typical of cowpox virus infections, in contrast to infections with so-called testicular or neuro-vaccines whose hemorrhagic zone on the periphery is sharply delineated from the intact surroundings and whose central necrosis leaves a deep crater in the more strongly developed infiltrate. Aside from local formation of inclusion bodies, whose unusual size in itself is pathognomonic of cowpox virus infections, the onward development of general symptoms is particularly severe in this disease. Secondary offlorescences appear on the depilated skin and on the mucous membranes; the animals are highly febrile and most of them succumb 10-14 days after infection. We never saw encephalitis after intracutaneous infection, as occasionally diagnosed by Herrlich & Mayr.

Based on this clearly expressed tendency to local hemorrhagic inflammation, the experienced observer can diagnose a cowpox virus infection intracutaneously. Generally speaking, this test will be used principally for quantitative determination of viral content or for neutralisation tests, although it must be pointed out that our own tests have yielded more exact values upon i.c. infection of mice (Dösch and Moritsch 1956).

Comparative studies of infections of the scarified cornea with cowpox or vaccinia virus lead Berger (1956) to the conclusion that the vehemence of reaction is less stormy with cowpox virus than with vaccinia virus, basing his assumption on the total diameter of the crater within the local lesion. The abnormal development of the size of inclusion bodies in the epithelial cells of the cornea, in comparison with vaccinia infections, was pointed out by Kaiser and Gherardini as early as 1940.

As in the case of vaccinia virus, the intracerebral infection of rabbits as a rule leads to encephalitis, although encephalitic lesions are regularly seen in our tests following intraocular infection, especially in the frontal lobe (Moritsch 1954). These vaccinia or cowpox-induced viral encephalitides of the rabbit are distinctly marked in histological specimens and differ fundamentally from the appearance of so-called postvaccinal encephalitis of man and from mice infected i.c. with cowpox virus. For this reason comparative morbid histological examinations had failed to produce positive results regarding the etiology of human postvaccinal encephalitis.

#### 4. Infection of the fertile, incubated hen's egg.

The chick fetus is the most common experimental animal used in laboratory diagnoses of viruses. Its diverse membranes with their variable infective possibilities permit differentiation of all strains of animal pox, as shown in comprehensive investigations by Herrlich and Mayr (1955). Since cowpox virus applied to the allantoic cavity asserts itself very poorly, most tests will rely on direct inoculation of the chorioallantoic membrane (CAM) and intravascular infection after Dösch (1954, 1956), since these methods promise optimal results.

In evaluating these infections of the hen's egg, a consistent survey will involve

- a) the time at which lesions appeared,
- b) the nature of individual defects, and
- c) the extent of secondary lesions on the membrane and on the whole fetus.

Direct inoculation of CAM with cowpox leads to appearance of first lesions at the site of infection after incubation for 20 hours, followed by the peak of the primary reproductive phase on the 3rd day. Embryos may die after application of high infective doses for this purpose. Otherwise 50% of the infected eggs show generalization after 70 to 82 hours as a sign of biphasic development, consisting of numerous secondary pox foci on the membrane and the fetus proper, causing death of all embryos. All of these infections were marked by rapid proliferative processes on the ectoderm and by relatively late central necrobiosis of these degenerate and proliferative epithelial cells. The vessels, as a rule, are infiltrated cellularly and consequently show marked thickening, resulting in defects of the vascular wall with consecutive, extensive extravasations. This hemorrhagic nature found on the egg membrane and in secondary foci may lead to erosion of larger vessels with resultant hemorrhages into the body cavities. The hemorrhagic nature of cowpox virus on the egg membrane is important with respect to differential diagnosis vis-a-vis testicular and neuro-lapines (vaccinia virus!), since the latter can never cause a hemorrhagic reaction on the egg membrane.

Generalization of the virus is expressed initially by secondary efflorescences on uninoculated parts of the CAM and, most pronounced, by enlargement of the liver, which is permeated by numerous necroses. However, there are no principal differences in the histological section between a direct infection and one caused indirectly by the hemal route. It must be remembered, however, that the primary lesions may already show regressive changes at the time of fetal death due to the time interval involved, which is readily explained by their "head start" of about 48 hours. When the intravascular technique of chick embryo infection is used according to Dosch (1954), phase 1 coincides with phase 2 and viral generalization is demonstrated throughout the egg after incubation for 2 days. The result is a local eruption at the site of injection, accompanied by uniformly disseminated, flatly lenticular tissue infiltrates along the blood vessels of the CAM, efflorescences of the yolk membrane and disseminated submiliary foci in the liver. The liver is enormously enlarged and degenerated; the foci themselves appear as focally necrotizing hepatitis with vascular ectasias.

This clinical condition may be survived by the fetus in phase 2 after direct inoculation of the CAM. Depending on the length of additional incubation, the lesions will show qualitative differences in the developmental stage, but a phase 3 of generalization has not been seen to date. However, when eggs are infected intravascularly according to Dosch (1954), the infective course may be followed by one additional phase which would coincide chronologically with phase 2 of direct CAM inoculation, and which this fetus would still survive, provided this mode of infection is utilized. This "phase 3" is manifested in contact infections of the fetal skin and the digestive and respiratory tract by hematogenically infected amniotic fluid. This particularly subtle infection enables the virologist to observe the course of an egg infected with a pox group



virus for extended periods of time, permitting inference of additional factors for the differentiation of individual virus strains.

The special criterion of cowpox infection, the tendency to form unusually large inclusion bodies, is demonstrated with ease in the ectodermal epithelial cells of the CAM. On the other hand, inoculation of the allantoic cavity with cowpox virus is rarely successful, in contrast to vaccinia virus, and produces only isolated cellular inclusions (Herrlich and Mayr 1955).

We must point out again that isolation of so-called "white mutants" is possible on the CAM; their peculiar behavior has been investigated extensively by van Tongeren (1952).

Finally, the characteristic and constant behavior of pox group viruses (including cowpox virus) in the fertile, incubated chick embryo offers possibilities of exact diagnosis and differential diagnosis among this group. Details have been summarized and recorded by Herrlich and Mayr (1955).

#### Definition of cowpox virus.

The consistent and characteristic behavior of cowpox virus in different animal species permits positive differentiation of this virus from other strains of the pox group, particularly from current vaccinia virus. The essential and principal peculiarities of cowpox virus are its tendency to form large inclusion bodies (especially in the epithelial cells of the ectoderm), the hemorrhagic nature of local inflammatory foci and the resulting augmented pathogenicity for the affected individual. Serologically, cowpox virus may be differentiated from variola and vaccinia virus, but not from ectromelia virus. A singular property is the formation of "white mutants" which differ virologically, but not serologically, from the original strain. The hypothetical assumption that this so-called "white mutant" may represent the starting material of our current vaccinia virus strains (Downie 1952) cannot be affirmed today and must await the results of exacter studies. In line with current scientific standards, the virus described here in detail must be designated "cowpox virus," and this nomenclature must be considered strictly separate and absolutely binding.